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TITLE:

Mercury Concentrations in Gafftopsail Catfish and Other Fishes in Waters Adjacent to Hobe Sound National Wildlife Refuge

ABSTRACT:

From September 22-26, 1990, 20 gafftopsail catfish (*Bagre marinus)* and 3 other fish species (n = 4) were collected from marine waters adjacent to Hobe Sound National Wildlife Refuge (NWR), Florida. The fish were collected for mercury analysis of muscle tissue. Ninety-five percent of the gafftopsail catfish (n = 19) had mercury levels that exceeded the Florida limited-consumption advisory of 0.5 parts per million (ppm), wet weight. Twenty-five percent (n = 5) exceeded Florida's no-consumption advisory of 1.5 ppm. Two other species, crevalle jack (*Caranx hippos*) (n = 2) and Atlantic croaker (*Micropogonias undulatus*) (n = 1), also had mercury concentrations exceeding the 0.5 ppm advisory. One black drum (*Pogonias cromis*) had a mercury concentration below advisory levels.

Evaluation of mercury/weight and mercury/length relationships of the gafftopsail catfish did not provide mechanisms by which recreational fishermen could selectively retain individual fish that are low in mercury.

The collection area between Hobe Sound and Peck Lake may be a marine environment that encourages bioconcentration of mercury in some marine fishes. Fish and wildlife trust resources may be at some risk when utilizing the food chain resources of these waters. Additional habitat and biota contaminant work is recommended.

KEY WORDS:

Mercury, blue crab, gafftopsail catfish, crevalle jack, black drum, Atlantic croaker, loggerhead sea turtle, wading birds, trust resources.

PREFACE

This report is written for the Fish and Wildlife Refuge System. However, we realize that much of the information contained in the report may be passed on to the general public by the Refuge Manager; therefore, the report is prepared in a "non-technical" format. English measurements are used throughout the report. We hope the format provides information for refuge personnel and recreational fishermen that can be easily and immediately understood. Metric measurements for the database (and their conversions to English units) can be found in Appendix D. In addition, we have used wet weight (fresh weight) values for mercury concentrations in all discussions about this metal. Wet weight values are consistently used by the State of Florida in setting consumption advisories. Sample dry weight values and tissue moisture values are presented in Appendix D.

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ACKNOWLEDGMENTS

Fish samples were collected with the assistance of Mr. Bob Jarvis, Biological Aide, Panama City Field Office. Logistical support was provided by Mr. Burkett S. Neely, Jr., Refuge Manager, Hobe Sound NWR and his staff.

We were assisted in report preparation and review by Dr. Charles Facemire, Region 4 Environmental Contaminants Coordinator, Ms. Kathy Hoffmaster, Office Automation Clerk, Mr. Hildreth Cooper, Biologist, and Ms. Gail A. Carmody, Panama City Field Office Project Leader.

INTRODUCTION

In September 1982, the Florida Game and Fresh Water Fish Commission began a survey of fishes in the Chipola River of northwest Florida to determine if contamination of fish had occurred. This action was prompted by pollution within the drainage basin of the Chipola River from a battery salvage plant located in Jackson County. Largemouth bass (*Micropterus salmoides*) collected from the Dead Lakes area of the Chipola River did have elevated mercury levels. To obtain "natural" background measurements for comparison, the "pristine" Santa Fe River was also chosen for sampling. Results were surprising. Elevated mercury levels were detected in Santa Fe River largemouth bass. These results led to the formation of an informal interagency task force composed of personnel from the Florida Game and Fresh Water Fish Commission, the Florida Department of Environmental Regulation, and the Florida Department of Health and Rehabilitative Services. Subsequently, a systematic statewide mercury investigation was initiated that involved the sampling of about 20 Florida lakes or streams each year. In 1988, the investigation revealed a mercury problem in largemouth bass and other species collected in the Everglades waterways of south Florida.

As a result of the State's mercury investigation, fish consumption health advisories were formulated by the Department of Health and Rehabilitative Services (HRS 1989) for largemouth bass and other species. The advisories recommend that when average concentrations of mercury (in the edible portion; i.e., fillet) are between 0.5 ppm and 1.5 ppm wet weight, adults should limit their consumption to no more than one meal of fish per week. Nursing mothers, women who are pregnant or anticipate bearing children, and children under 15 years of age are advised not to eat these fish more than once a month. Fish that contain more than 1.5 ppm of mercury should not be eaten by anyone (HRS 1989). Approximately one million acres of the Everglades and another one million acres of Florida freshwater areas have been posted with advisories (Lambou, et al. 1991).

Because of the large area of National Wildlife Refuge (NWR) lands in Florida not previously included in the State investigation, the U.S. Fish and Wildlife Service (Service) sampled selected refuges in Florida to determine mercury levels in the fish within these refuges. Many federal trust resource species utilize the Florida refuges, including endangered species, migratory birds, and anadromous fishes.

The objectives of the NWR studies were to determine if fish had levels of contamination that would trigger a consumption advisory and which might also significantly affect individuals or populations of fish and wildlife resources under refuge management.

The field work for this investigation was done between September 22-26, 1990. The investigation involved the collection of upper trophic level predator fish species, the analysis of muscle tissue from those fish, and an evaluation of the data as it related to length and weight of the fish collected.

Investigations into the identification of specific mercury sources, the mechanisms of mercury transport and deposition, and the dynamics of mercury biotransformation and biomagnification within biota were beyond the scope of this study.

MERCURY

Mercury (Hg) and its compounds do not have any known normal metabolic function. The presence of mercury in cells of living organisms represents contamination from natural and/or anthropogenic sources. Any such contamination should be regarded as undesirable and potentially hazardous (Eisler 1987). Additional information about the nature of mercury is provided in Appendix A.

SITE DESCRIPTION

Hobe Sound National Wildlife Refuge (NWR) (Figure 1) includes three and one-half miles of beach, sand dunes, and mangroves on Jupiter Island and a sand scrub forest on the mainland. The Refuge was established on September 30, 1969, when residents of Jupiter Island donated 229 acres to the U.S. Fish and Wildlife Service. Additional donations have brought the total to 968 acres. Today the Service manages the Refuge to provide habitat and protection for a variety of wildlife native to this coastal area.

Hobe Sound NWR is one of the most productive sea turtle nesting areas in the United States and provides prime nesting area for the endangered leatherback turtle (*Dermochelys coriacea*) and green sea turtle (*Chelonia mydas*); and the threatened loggerhead sea turtle (*Caretta caretta*). The loss of nesting beaches to coastal development has been a major factor in the decline of these species. In good years, over 100,000 turtle hatchlings may be produced along the Refuge's three and one-half miles of beach.

Other federal or state endangered, threatened, and special-concern species that either live on, or utilize, Refuge resources include the bald eagle (*Haliaeetus leucocephalus)*, eastern indigo snake (*Drymarchon corais couperi*), scrub jay (*Aphelocoma coerulescens*), gopher turtle (*Gopherus polyphemus*) and West Indian manatee (*Trichechus manatus*).

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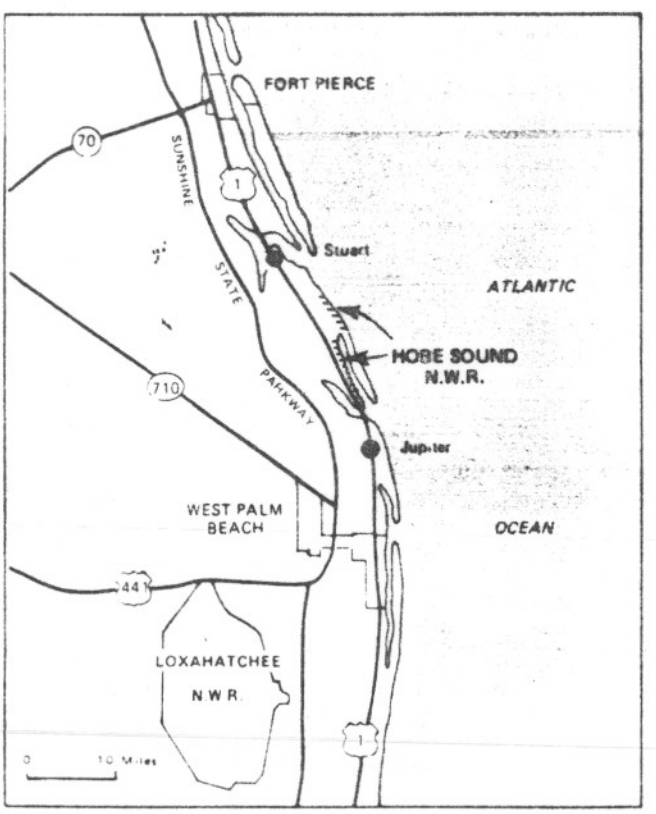
Figure 1. Location of Hobe Sound National Wildlife Refuge



Migratory bird trust resources include the brown pelican (*Pelecanus occidentalis*), osprey (*Pandion haliaetus*), and a variety of shore birds that are abundant along the ocean beach and mudflats of the Intracoastal Waterway. In addition, wading birds feed in the mangrove swamps and mosquito-control impoundments. A variety of songbirds live in the sand pine scrub forest.

Resident mammal species living on the Refuge include white-tailed deer (*Odocoileus virginianus*), raccoon (*Procyon lotor*), gray fox (*Urocyon cinereoargenteus*), and bobcat (*Lynx rufus*).

Threats to the habitats and resources of this south Florida Refuge include global and regional air pollution, stormwater runoff from residential and business development, industrial activities with their associated runoff and point-source discharges, extensive recreational boat and commercial vessel traffic, regional agricultural operations, and insect control programs.

SAMPLING AREA

Samples of fish were collected from one area that extended from Hobe Sound north to Peck Lake (Figure 2). Collections took place over unvegetated sediments. Average water depth was approximately six to ten feet.

MATERIALS AND METHODS

Gafftopsail catfish (*Bagre marinus)* measuring 16 inches or more (total length) were retained for analysis. Fish were collected in monofilament gill nets 150 feet long, 8 feet deep, and made of three 50-foot panels; each with equal areas of 2, 2.5, and 3-inch square mesh netting. The nets were equipped with foamcore float line and #30 leadcore lead line. Individual nets were anchored and marker-buoyed at each end. Nets were fished for four-hour periods and checked for specimens.

Collected specimens were removed from the nets and immediately placed on ice in clean thermal containers. Fish samples were prepared and stored in accordance with standard operating procedures for the collection of fish tissue samples (PCFO-EC-SOP-001, 1988), Appendix B. All samples were placed in a field freezer and frozen within eight hours of collection.

Upon returning to Panama City, samples were transferred to a storage freezer maintained at -10 degrees Fahrenheit. Samples were shipped to analytical laboratories after approximately 120 days of freezer storage. Laboratory protocols for handling and analysis of mercury are found in Appendix C. Appendix D contains the study data.

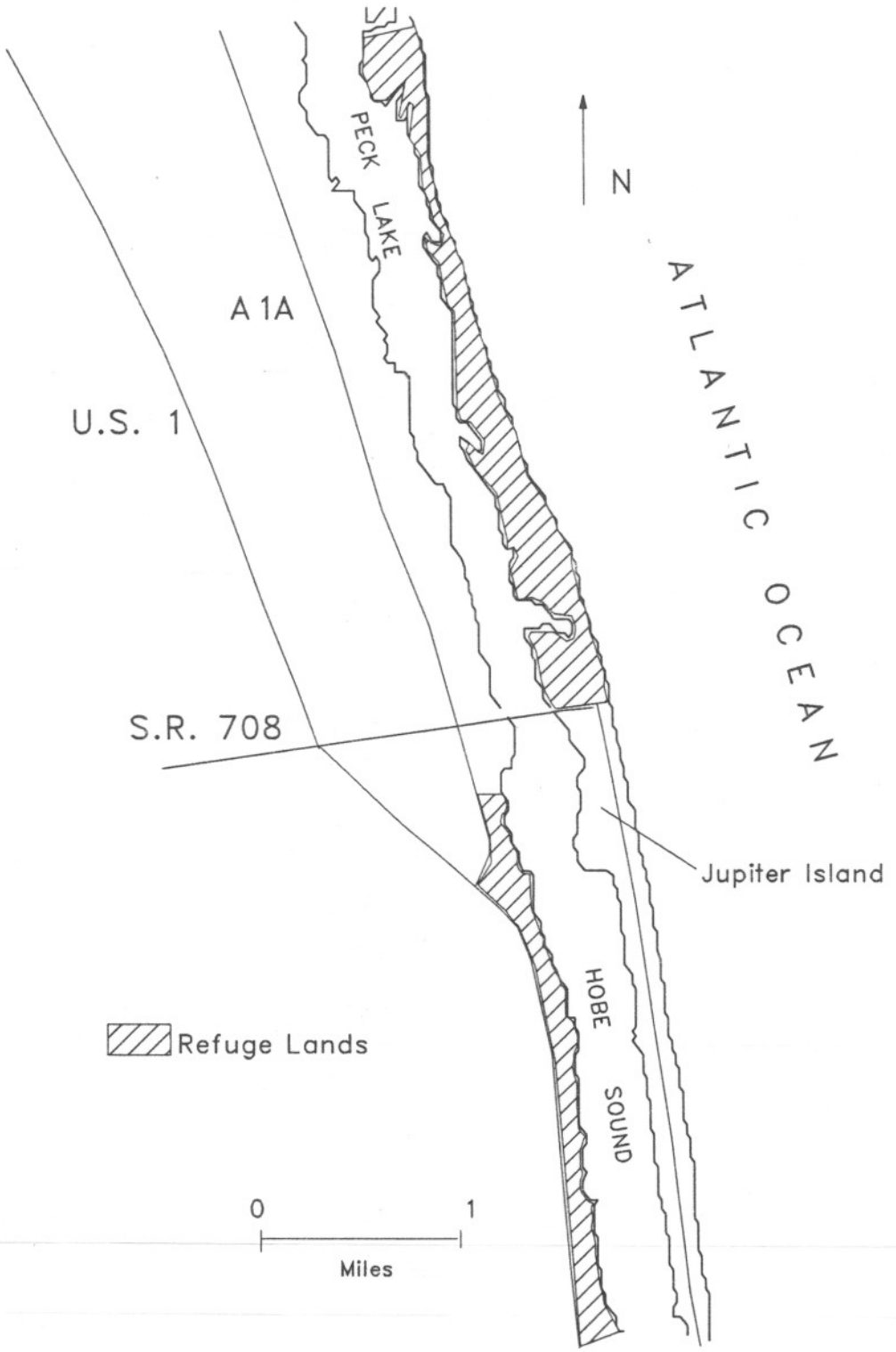


Figure 2. Hobe Sound NWR Fish Collection Area

The fish mercury concentrations and morphometric measurements were statistically evaluated after the data for mercury concentrations were log-transformed to meet criteria for normal distribution. Correlation analysis was used to determine the degree of relationship between certain morphometric variables and mercury concentrations (Sokal and Rohlf 1969).

RESULTS

Table 1 presents the results of the field collections in the marine waters adjacent to Hobe Sound NWR. Four species of fish were collected: gafftopsail catfish, crevalle jack, black drum, and Atlantic croaker.

MERCURY IN GAFFTOPSAIL CATFISH

Twenty gafftopsail catfish were analyzed for mercury in muscle tissues (i.e., fish fillets). Ninety-five percent (n = 19) had mercury exceeding the Florida lower-level consumption advisory of 0.5 ppm mercury, wet weight. Twenty-five percent (n = 5) exceeded the upper-level consumption advisory of 1.5 ppm.

Table 1. Fish collections from marine waters adjacent to the Hobe Sound NWR, September, 1990.

Sampling Location	Spp.	# of Ind.	Length: average (range)	Weight: average (range)	Mercury:[c] average (range)
Hobe Sound	GTC	20	21[a] (16-25)	62[b] (18-113)	1.13[d] 1.04[e] (.47-2.13)
Hobe Sound	CJ	2	13.5 (13-14)	21 (20-22)	.64 .63 (.56-.71)
Hobe Sound	AC	1	14 (-)	13 (-)	.82 (-)
Hobe Sound	BD	1	14 (-)	29 (-)	.19 (-)
Species Codes: GTC/gafftopsail catfish CJ/crevalle jack AC/Atlantic croaker BD/black drum					

[a] Length = inches
[b] Weight = ounces
[c] Parts per million, wet weight
[d] arithmetic mean
[e] geometric mean

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Mercury and Total Weight of Gafftopsail Catfish

The gafftopsail catfish data were sorted by pound classes (Figure 3). Only two-pound classes had five or more individuals per group. These classes were Class III (from 3 to 4 pounds) and Class IV (from 4 to 5 pounds). Inspection of these classes shows that mercury concentrations varied within each class from approximately 0.5 ppm to 1.5 ppm. In Classes V and VI (fish five pounds or larger), all individuals exceeded a mercury concentration of 1.5 ppm.

The data in Figure 3 suggest that almost any gafftopsail catfish within the collection area, weighing one pound or more, would have a mercury muscle concentration greater than 0.5 ppm, and that many individuals would exceed 1.0 ppm.

Given the variation in mercury concentrations exhibited in Classes III and IV, it would appear that no strong association exists between the total weight of a catfish and the amount of mercury it contains in its muscle tissue. To test this hypothesis, the data were subjected to correlation analysis. The resulting product-moment correlation coefficients (wet weight values, $r = 0.649$, $p > 0.01$; dry weight values, $r = 0.645$, $p > 0.01$) support this hypothesis. Calculation of the coefficients of determination (r^2) reveal that only about 42% of the variance in mercury concentrations in muscle tissue is accounted for by the variation in the fish's total weight. Figure 4 is a plot of the individual weight of each fish. It illustrates the variation within weight groups.

Mercury and Total Length of Gafftopsail Catfish

The gafftopsail catfish were also sorted by their total lengths (in inches - Figure 5). Total length was evaluated as a potential tool for roughly estimating, in the field, the amount of mercury that might be in an individual fish.

Generally, the same loose pattern of association can be observed. Five individuals were 21 inches long. Another five fish were 23 inches long. The 21-inch fish had mercury concentrations that varied from about 0.5 ppm up to 1.5 ppm. The 23-inch fish had concentrations that varied from about 1.0 ppm to over 2.0 ppm. In each of these two length groups mercury concentrations (among individuals) had a range covering a full part per million.

When the mercury and total length data were subjected to correlation analysis, the product-moment correlation coefficients were as follows: wet weight values, $r = 0.654$, $p > 0.01$; dry weight values, $r = 0.661$, $p > 0.01$. Calculation of the coefficients of determination (r^2) revealed that, again, only about 43 to 44% of the variance in mercury concentrations in muscle tissue is accounted for by the variation in fish length.

Figure 6 is the frequency distribution for the Hobe Sound gafftopsail catfish.

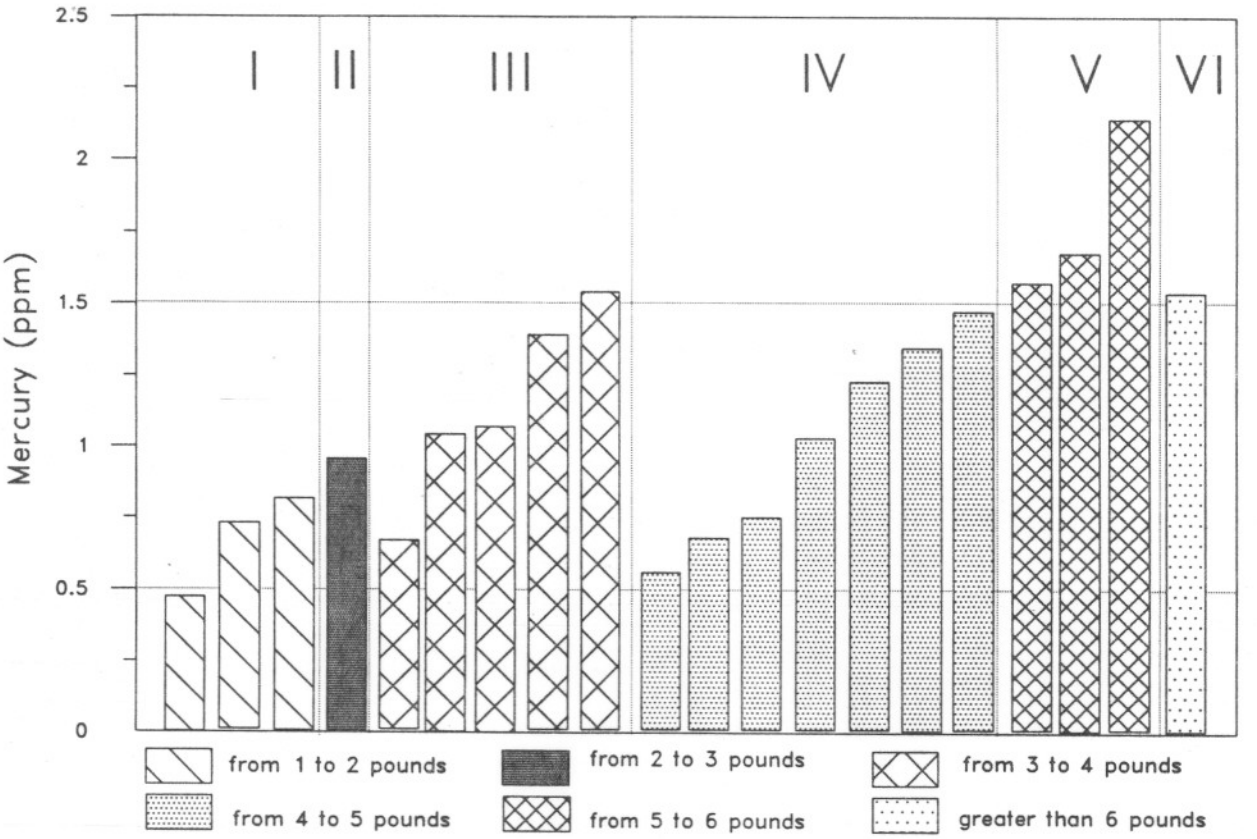

Figure 3. Variation of mercury concentration in gafftopsail catfish by pound class, Hobe Sound NWR, 1990.

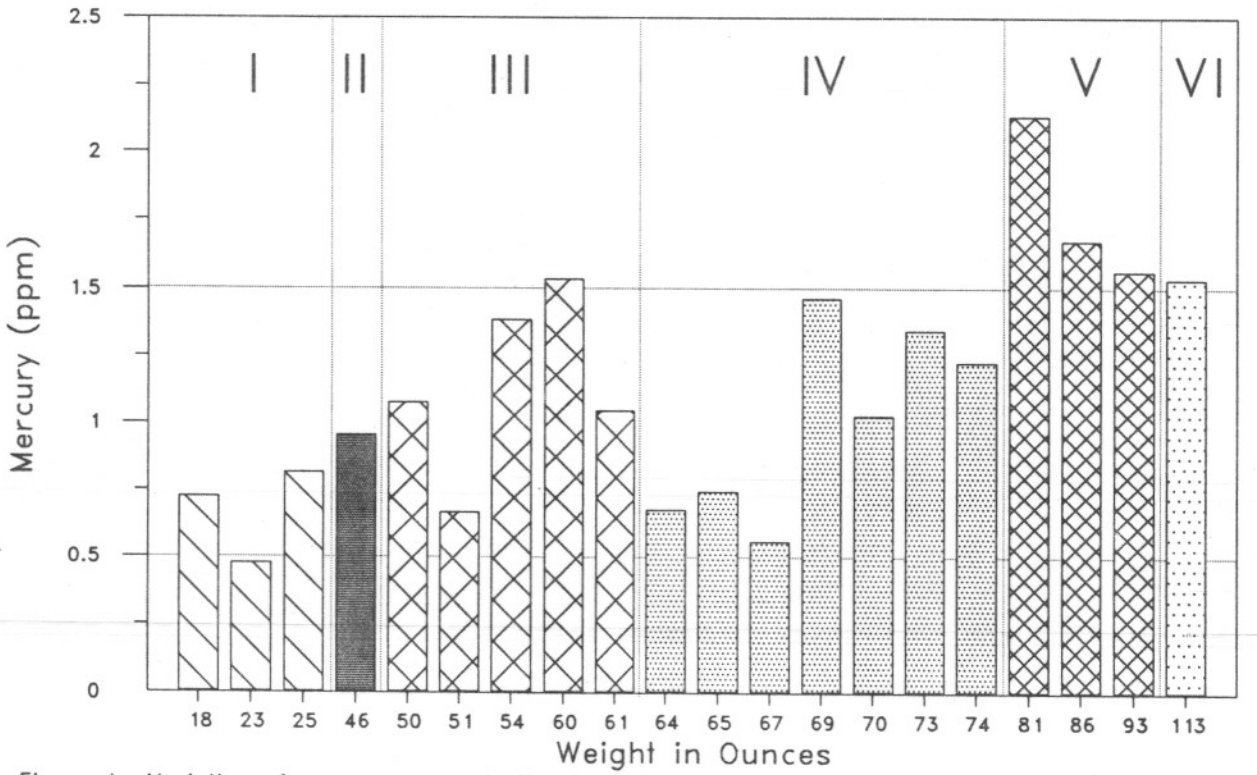

Figure 4. Variation of mercury concentration by weight, in ounces, Hobe Sound NWR, 1990.

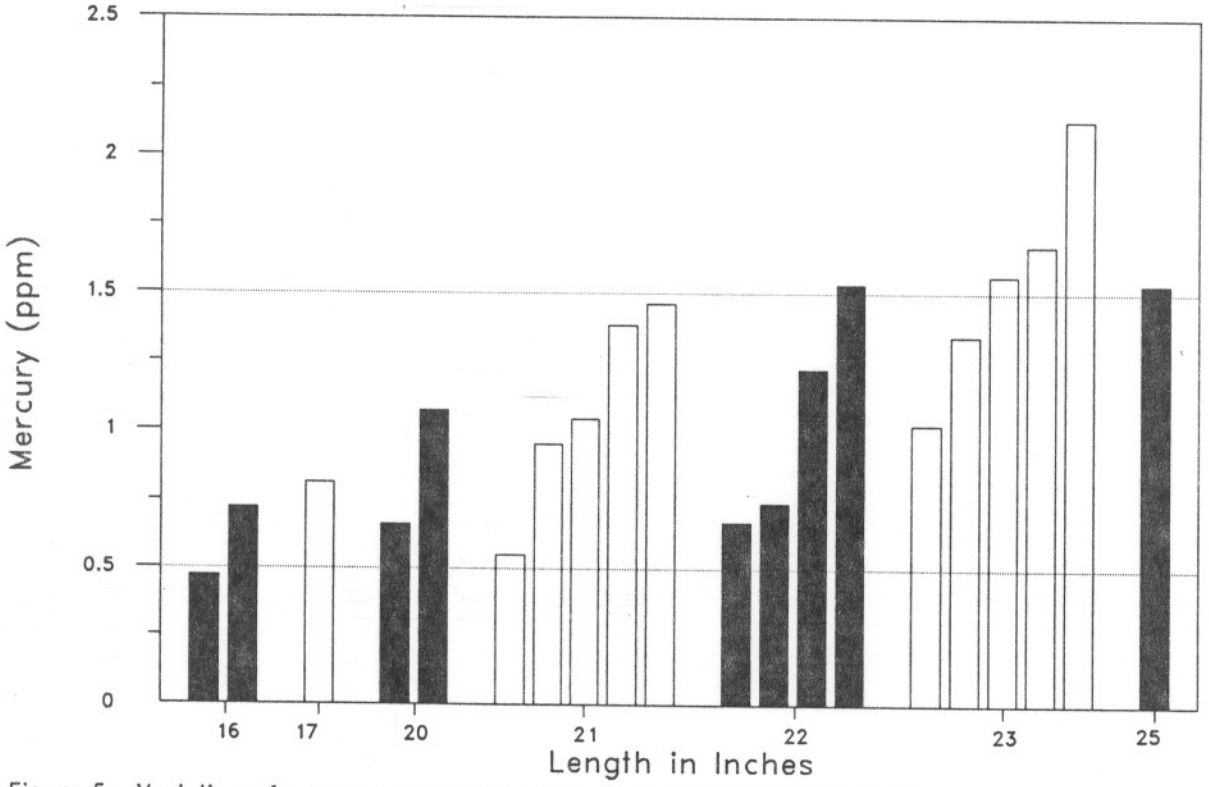

Figure 5. Variation of mercury concentration in gafftopsail catfish by inches, Hobe Sound NWR, 1990.

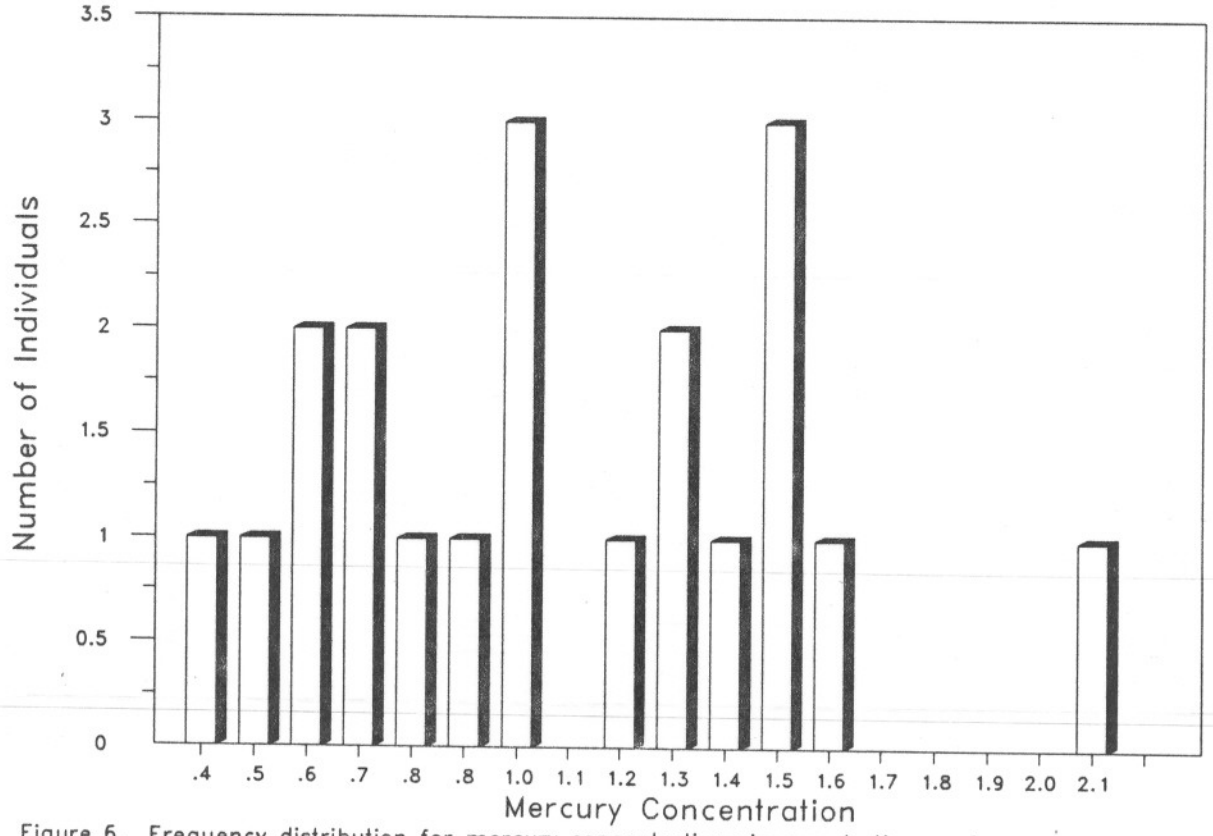

Figure 6. Frequency distribution for mercury concentrations in muscle tissue of gafftopsail catfish collected in Hobe Sound, 1990.

OTHER FISH SPECIES

Crevalle Jack (*Caranx hippos*): Mercury in Muscle Tissue

Two crevalle jacks were collected in Hobe Sound. These fish were 13 inches and 14 inches long. They weighed 20 and 22 ounces, respectively. The wet weight mercury concentrations were 0.56 and 0.71 ppm respectively in their muscle tissue. Even though the larger crevalle jack was only 7% longer and 9% heavier than the smaller individual, the larger fish had 27% more mercury in its tissues.

Sciaenid Fishes (Drum Family): Mercury in Muscle Tissue

One Atlantic croaker (*Micropogonias undulatus*) and one black drum (*Pogonias cromis*) were collected. The croaker was 14 inches long and weighed 13 ounces. The drum was also 14 inches long, but it weighed 29 ounces. The mercury concentrations in the muscle tissue were 0.82 ppm for the Atlantic croaker and only 0.19 ppm for the much heavier black drum.

DISCUSSION

The evaluation of gafftopsail catfish collected from marine waters adjacent to the Hobe Sound NWR revealed that all individuals contained some mercury in their muscle tissue, and that many contained concentrations ranging between the State advisory concentrations of 0.5 and 1.5 ppm wet weight (Figure 6).

For the gafftopsail catfish collected in this study, no strong relationship was observed between either total length or total weight of these fish and the amount of mercury in muscle tissue. Therefore, there appears to be no simple, convenient way for recreational fishermen or biologists to estimate the approximate amount of mercury in any individual gafftopsail catfish based on these physical characteristics.

Gafftopsail catfish were used as a general indicator of the availability of mercury to fish and wildlife in the region of the Florida coast where the Hobe Sound NWR is located. While these catfish are thought to avoid lower winter water temperatures in most estuaries by migrating offshore in winter, at least three studies infer that adult gafftopsail catfish remain year-round in southern Florida inshore waters (Gunter and Hall 1965; Tabb and Manning 1961; Roessler 1970) because of mild, year-round temperatures.

Several authors (Gudger 1916; Gunter 1945; Darnell 1961; McClane 1965; Gallaway and Strawn 1974) found that blue crabs are a principal food item of gafftopsail

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catfish. These crabs are also consumed by loggerhead sea turtles. In at least one survey (U.S. Fish and Wildlife Service, 1986) four composite samples (n = 5) of blue crabs from St. Andrew Bay, Florida had whole-body, wet weight mercury concentrations of 0.14, 0.11, 0.16, 0.08 ppm. In view of the mercury found in the Hobe Sound catfish, this toxic metal may pose a threat to the loggerhead sea turtle. The turtles may be exposed to mercury through food chain organisms such as the blue crab that inhabit the Hobe Sound area.

Smaller size forage fish also accumulate mercury, but usually to lesser degrees than top carnivore fish. Composite samples (n = 10) of spot (*Leiostomus xanthurus*), Atlantic croaker, and silver perch (*Bairdiella chrysoura*) collected from St. Andrew Bay had whole-body, wet weight mercury concentrations of 0.02 to 0.07 ppm mercury (U.S. Fish & Wildlife Service, 1986). Wading birds feed on these fishes and other forage species. If local fish populations contain mercury, bioaccumulation can occur in the birds consuming them.

Although this study was limited primarily to the analysis of fish muscle tissue, other body tissues represent varying sources of mercury that are available to wildlife. The partitioning of mercury in other body compartments of fish is illustrated for two species in Table 2.

Table 2. Partitioning of mercury in some body compartments of two fish species. Fish were collected in St. Andrew Bay, Florida (spotted sea trout), Apalachicola River, Florida, and Flint River, Georgia (striped bass). Mercury values are ppm wet weight. USFWS data, Panama City, Florida

Species	Muscle	Liver	Offal*	Fat	Gonad
Spotted Sea Trout	0.40	0.33	0.27	-	0.08
Spotted Sea Trout	0.56	0.28	0.26	-	0.10
Spotted Sea Trout	0.48	0.24	0.25	-	0.04
Striped Bass	0.45	0.37	-	0.06	0.06
Striped Bass	0.68	0.78	-	0.17	0.15
Striped Bass	0.40	0.24	-	0.02	-

*** Offal.** The waste or byproduct of a process. In this case, the remainder of each fish after fillets (muscle tissue), liver, mesentery fat, and gonads had been removed.

The Table reveals that considerable amounts of mercury accumulate in the liver and other body parts. Not as much mercury accumulates in fat and reproductive tissue. Wildlife feeding on fish in the areas where our collections took place may be building up mercury in their body tissues. Species of particular concern include the bald eagle, wood stork, osprey, anhinga, and various species of fish-eating wading birds.

CONCLUSIONS AND RECOMMENDATIONS

The mercury concentrations in fish tissue samples (edible fillets) from the study area often exceeded State consumption advisory levels. It is possible that sea turtles and certain species of migratory birds feeding in waters near the Refuge may be accumulating undesirable amounts of mercury.

The following actions are recommended as a result of this study:

1) Further investigation of the Hobe Sound National Wildlife Refuge environment.

2) Additional sampling and chemical analysis of trust resource species and their food chain organisms.

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APPENDIX A

THE NATURE OF MERCURY

Mercury (Hg) and its compounds have no known normal metabolic function. The presence of mercury in cells of living organisms represents a contamination from natural and/or anthropogenic sources. Any such contamination should be regarded as undesirable and potentially hazardous (Eisler 1987).

Some forms of mercury with relatively low toxicity can be transformed into forms with very high toxicity through methylation and other biological processes. Methyl mercury can be bioconcentrated in organisms and biomagnified through food chains, returning mercury directly to man and other upper trophic level consumers in concentrated form. Mercury has mutagenic, teratogenic and carcinogenic properties, and has caused embryocidal, cytochemical and histopathological effects. High body burdens of mercury normally encountered in some species of fish and wildlife from remote locations emphasize the complexity of natural mercury cycles and human impact on these cycles. Some scientists believe that the anthropogenic release of mercury into the environment should be curtailed because the difference between tolerable natural background levels of mercury and harmful effects in the environment is exceptionally small (Eisler 1987).

Mercury from natural sources can enter the biosphere as a gas from terrestrial and oceanic volcanic activity, in solution, or in particulate form. Cinnabar (HgS) is a common mineral in hot springs deposits and a major natural source of mercury. The global cycle of mercury involves degassing of the element from the earth's crust, evaporation from natural bodies of water, atmospheric transport (mainly in the form of mercury vapor), and deposition of mercury back onto land and water. Oceanic effluxes of mercury are tied to equatorial upwelling and phytoplankton activity and may significantly affect the global cycling of this metal. If volatilization of mercury is proportional to primary production in the world's oceans, oceanic phytoplankton activity represents about 36 percent of the yearly mercury flow to the atmosphere (Eisler 1987).

Human activities that contribute significantly to the global input of mercury include the combustion of fossil fuels, mining and reprocessing of gold, copper, and lead, operation of chloralkali plants, and disposal of batteries and fluorescent lamps. The production of electrical apparatus, industrial control instruments (switches, thermometers, and barometers, etc.), laboratory appliances, anti-fouling and mildew-proofing paints, chemical formulations to control fungal diseases of seeds, bulbs, and

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vegetables, dental amalgams, pulp and paper, pharmaceuticals, and metallurgy and mining, is contributing, or has contributed, mercury to the environment (Eisler 1987).

Mercury burdens in sediments and other non-biological materials are estimated to have increased up to five times prehuman levels; primarily as a result of man's activities. The estimated half-time residence value for mercury is comparatively short in the atmosphere, between 6 and 90 days, but is much longer in terrestrial soils, oceanic waters, and oceanic sediments where it is estimated to remain 1,000, 2,000 and more than one million years, respectively (Eisler 1987).

An elevated concentration of mercury (usually as methyl mercury) in any biological sample is often associated with proximity to human use of mercury. The elimination of mercury point-source discharges has usually been successful in improving environmental quality. However, elevated levels of mercury in biota may persist in contaminated areas long after the source of pollution has been discontinued. It is noteworthy that some groups of organisms with consistently elevated mercury residues may have acquired these concentrations as a result of natural processes, rather than from anthropogenic activities. These groups include older specimens of long-lived predatory fishes, marine mammals (especially seals and sea lions), and organisms living near natural mercury ore/cinnabar deposits.

Certain species of macrophytes strongly influence mercury cycling. For example, saltmarsh cordgrass (*Spartina alterniflora*), a dominant salt marsh plant in Georgia estuaries -- accounted for almost half the total mercury budget in that ecosystem (Eisler 1987). Mangrove vegetation plays a similarly important role in mercury cycling in the Florida everglades (Eisler 1987). These findings suggest that more research is needed on the role of higher plants in the mercury cycle. In aquatic ecosystems, removal of the source of anthropogenic mercury results in a slow decrease in the mercury content of sediments and biota. The rate of loss depends, in part, on the initial degree of contamination, the chemical form of the mercury and the half-life of that form, physical and chemical conditions of the system, and the hydro-dynamics of the particular aquatic ecosystem.

Methyl mercury is produced by methylation of inorganic mercury present in both freshwater and saltwater sediments, and accumulates in aquatic food chains in which the top level predators usually contain the highest concentrations (Eisler 1987). Most organomercury compounds other than methyl mercury decompose rapidly in the environment, and behave much like inorganic mercury compounds (Eisler 1987). In organisms near the top of the food chain, such as carnivorous fishes, almost all mercury accumulated is in the methylated form, primarily as a result of the consumption of prey containing methyl mercury. A strong relationship appears to exist between elevated mercury in Florida largemouth bass and low pH waters from

swamp or peat drainage. A negative correlation exists in Florida for highly eutrophic waters (enriched), where depressed mercury levels are typically found.

Methylation also occurs within the biological organisms themselves because intestinal bacteria convert mercury into methyl mercury through enzymatic processes. However, this methylation process, as a mercury uptake source, is not as important as intake of methyl mercury via the animal's diet.

There is no known effective antidote to counteract the effects of methyl mercury poisoning on the vertebrate central nervous system (Eisler 1987). Mercury binds strongly with sulfhydryl groups and has many potential target sites during embryogenesis. Phenyl mercury and methyl mercury compounds are among the strongest inhibitors of cell division (Eisler 1987). Organomercury compounds, especially methyl mercury, cross placental barriers and can enter mammals by way of the respiratory tract, gastrointestinal tract, skin or mucus membranes (Eisler 1987). Compared with inorganic mercury compounds, organomercurials are more completely absorbed, or more soluble in organic solvents and lipids, pass more readily across biological membranes, and are slower to be excreted (Eisler 1987).

Mercury, at comparatively low concentrations, adversely affects the reproduction, growth, behavior, metabolism, blood chemistry, osmoregulation, and oxygen exchange of marine and freshwater organisms (Eisler 1987). In general, the accumulation of mercury by aquatic biota is rapid, and depuration is slow. Organomercury compounds, especially methyl mercury, have been found to be significantly more effective than inorganic mercury compounds in producing adverse effects and accumulations. Adverse affects of mercury to aquatic organisms have been documented at water concentrations of 0.88 to 5.0 ug/l. Enzyme disruption occurred in brook trout (*Salvenlinus fontinalis*) embryos exposed for 17 days in solutions containing 0.88 ug/l of methyl mercury (Eisler 1987). Increased incidence of frustule abnormalities and burst thecae were documented in two species of marine algae exposed to 1.0 ug/l concentrations of Hg^{++} for 24 hours (Eisler 1987). Arrested development of sea urchin larvae occurred in a 40-hour test when the larvae were exposed to 3.0 ug/l concentrations of Hg^{++} (Eisler 1987). Decreased rate of intestinal transport of glucose, fructose, glycine, and tryptophan occurred in the murrel (*Channa punctatus)* when exposed to 3.0 ug/l concentrations of Hg^{++} for 30 days (Eisler 1987). The blood chemistry of striped bass (*Morone saxitalis*) was altered when these fish were exposed to 5.0 ug/l concentrations of Hg^{++} for 60 days (Dawson 1982). Decreased respiration in striped bass was observed 30 days post exposure after immersion for 30 to 120 days in 5.0 ug/l concentrations of Hg^{++} (Eisler 1987).

STANDARD OPERATING PROCEDURES FOR COLLECTION OF FISH TISSUE SAMPLES

Fish collected for chemical contaminant evaluations may be taken by electrofishing gear, monofilament gill nets, otter trawl, haul or beach seines, fish traps, trotlines, or rod and reel. However, any collecting gear should be free of chemical treatments and/or metals that could contaminate samples. This is particularly important when the entire fish (whole body analysis) will be used.

For species of special concern such as Gulf sturgeon or large broodstock striped bass, we utilize only incidental mortalities, and these should be fresh specimens.

The following is a sample dissection.

1. Wash hands thoroughly and rinse completely. Wear vinyl or latex gloves. Final rinse with distilled water.

2. Fish should be clean. It may be rinsed of debris or mud in the waters of the collection site.

3. The dissection surface (work area) should be a chemically inert substance such as a stainless steel acetone-rinsed pan, or counter. Avoid letting the dissected sample touch this surface, if possible.

4. Use previously cleaned, and acetone-rinsed, then distilled water-rinsed stainless steel dissection tools (knives, scalpels, etc.). Scales for total fish weights and sample weights should also be clean or covered with pre-cleaned aluminum foil. Measuring devices for fish lengths, etc., should be clean, or should not come in contact with the specimen.

5. Do not let dissected samples remain exposed to the air. Exposure can dry samples and reduce the natural percentage of moisture. Prepare each dissected sample for shipping or freezing as it is dissected.

6. Samples should be placed in the smallest, pre-cleaned glass jar that will adequately hold the sample. The jars should be pre-labeled with a permanent, waterproof marking pen on the outside of the jar. Jars should also have a teflon liner inside the lid. As an alternative, acetone-rinsed, heavy-duty aluminum foil may be used to wrap the sample. After double-wrapping, place the sample (with sample identification label) inside an air-tight zip-lock bag.

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The environmental cycle of mercury is delicately balanced and small changes in input rates, and/or the chemical forms of mercury, may result in increased methylation rates in sensitive systems. For example, the acidification of natural bodies of freshwater is statistically associated with elevated concentrations of methyl mercury in the edible tissues of predatory fishes. In chemically sensitive waterways such as poorly buffered lakes, the combined effects of acid precipitation and increased emissions of mercury to the atmosphere (with subsequent deposition) pose a serious threat to the biota if optimal biomethylation conditions are met.

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MP-HGE-MA
PAGE: 1 OF 10
DATE: 01/17/92
REPLACES: Original
SECTION: 6005

ASSAY TITLE: Mercury

AREA OF APPLICABILITY: Hazleton Wisconsin, Inc.
Atomic Absorption

SCOPE:

This method is applicable to most materials including feeds, animal tissues, plants, soils, and food products.

PRINCIPLE:

Samples are digested with a mixture of sulfuric and nitric acids. Mercury is reduced with sodium borohydride for determination. The amount of mercury is determined at a wavelength of 253.7 nm by comparing the signal of the unknown sample (measured by the atomic absorption spectrophotometer with the MHS-20 hydride generation unit) with the signal of the standard solutions.

SENSITIVITY, PRECISION, AND ACCURACY:

The precision and accuracy of this assay have not been determined. Using a 2.0 g sample, the lowest detection limit of this assay is 0.025 ppm.

REFERENCE:

"Test Methods for Evaluating Solid Waste," EPA Publication No. SW-846, Second Edition, Methods 3030, 3040, or 3050; and 7470, U.S. EPA, Washington, D.C. (Revised April 1984).

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7. Sample identification labels should be prepared with permanent, waterproof ink or other writing instruments that will not bleed out or wash out, and should provide the following information:

   a. species name and common name,
   b. type of tissue (if not whole body),
   c. collection location,
   d. latitude and longitude,
   e. county and state,
   f. weight of sample in grams,
   g. date of collection,
   h. sample collector's name,
   i. total weight of fish specimen (grams),
   j. total length and fork length of specimen (cm), and
   k. method of collection.

8. Samples should be frozen as soon as possible. If samples contain large amounts of liquids that may expand, the lids may be set on the jars, without securing, until the sample has expanded and frozen. Then lids should be secured tightly.

9. Photographs of the specimens are desirable, as well as a written description of any external or internal lesions, tumors, etc.

APPENDIX C

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APPROVED BY: John L. Walton DATE: 1-10-92
John Walton
Staff Scientist/Section Leader
Inorganic Environmental Analysis

David C. Hills DATE: 1-14-92
David C. Hills
Manager
Inorganic Environmental Analysis

REVIEWED BY: Sherry R.W. Petsel DATE: 1-17-92
Sherry R.W. Petsel
Manager
Quality Assurance Unit

(1518D)

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SAFETY PRECAUTIONS:

- Mercury and its salts are highly toxic. Use extreme care when handling mercury solutions and wash hands thoroughly afterward.
- When using sodium borohydride, sulfuric acid solutions, or sulfuric acid:nitric acid mixtures, avoid contact with skin and inhalation of vapors because these solutions and their vapors are corrosive. If any of these solutions come into contact with skin, immediately flush the skin with running water for at least 10 minutes.
- Handle potassium permanganate cautiously. Diluted solutions are mildly irritating and high concentrations are caustic.
- Observe all standard laboratory safety procedures as outlined in the Hazleton Wisconsin Safety Training Manual.

FUNDAMENTAL EQUATIONS:

$$Hg^{2+} + 2BH_4^- \text{ -----> } Hg + H_2 \uparrow + 3/2\ B_2H_6$$

INTERFERENCES:

No known interferences exist.

QUALITY ASSURANCE:

Strictly follow the following requirements to avoid possible contamination.

- Closely match the concentration of reagents in both samples and standards because acidity and viscosity affect instrument sensitivity.
- Include a reagent blank with every run. Take it from the first step of sample preparation through the actual analysis.
- Include a duplicate sample with every run (ideally at a frequency of approximately 5% to 10%) and take it from the first step of sample preparation through the actual analysis.
- Include a recovery as appropriate and take it from the first step of sample preparation through the actual analysis.

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- Include a digestion control or a validated control sample (if available) as appropriate and take it from the first step of sample preparation through the actual analysis.
- Use deionized water and acid-washed glassware.
- Ensure that the sample is completely digested. Contact the area supervisor with any problems.
- Refer to the Standard Operating Procedures (SOPs) for Inorganic Analysis.
- See the Instrument Operating Procedure (IOP) for the appropriate atomic absorption spectrophotometer and hydride generation unit.
- Clean and rinse all tubing and cells before a day's run by running deionized water and then methanol through all components and drying them with a flow of nitrogen.
- Test the sulfuric acid and potassium permanganate solutions for possible mercury contamination before use.
- Consider a blank greater than the lowest standard to be significant and take it into consideration according to the SOPs for Inorganic Analysis.

APPARATUS:

- Round-bottomed flasks, 300 mL with two short necks, carrying 35/25 center and 18/9 side-ground glass joints
- Water condensers, 2.5 cm in diameter, 30 cm long, 35/25 ground glass balloon bottom
- Heating mantles, 335 watts, 300 mL, with a continuous heat variance
- Volumetric flasks, Class A, acid-washed
- Pipettes, Class A
- Mercury hollow cathode lamp or electrodeless discharge lamp
- Analytical balance, ±0.01 g for samples, ±0.0001 g for standards and reagents
- Magnetic stir plate
- Variac to control digestion temperature
- Glass stopper with 18/9 ground glass fitting

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- Rack for holding digestion apparatus, including clamps for condensers, rack for mantles, and electrical socket apparatus (four female sockets with one male plug) for heating mantles
- Cold water source and tubing to run condenser system
- Boiling chips, acid-washed, 50 mL
- Erlenmeyer flasks for storing reagents
- Graduated cylinder, acid-washed
- Atomic absorption spectrophotometer
- Hydride generation unit, including appropriate quartz cell for vapor analysis and appropriate tubing

Note: Equivalent equipment may be substituted.

REAGENTS:

- 1,000 ppm mercury stock solution, Fisher certified or equivalent.
- Mercury working standard: Recommended concentration is 1 ppm in 20% H_2SO_4. Prepare every 3 months.
  - Serial dilute stock solution by factors of 10 to 1 ppm in 100 mL in 20% H_2SO_4.
- Mercury working standards: Recommended concentrations are 0.01 and 0.001 ppm in 20% sulfuric acid, preserved with one drop of potassium permanganate solution. Prepare both on each day of use by serial dilutions of the 1 ppm working standard.
- 3% sodium borohydride in 1% sodium hydroxide: Dissolve 3.0 g sodium borohydride and 1.0 g sodium hydroxide in 100 mL deionized water. When in solution, vacuum filter the mixture using a 0.45-micron filter disc. Prepare fresh solution each day of use to avoid decomposition and liberation of hydrogen.
- Potassium permanganate saturated solution: Weigh 8.0 g of potassium permanganate into a 200-mL volumetric flask, add deionized water to dissolve it, fill the flask to volume, and mix the solution by inversion.
- Concentrated nitric acid, AR grade (marked and kept separate for mercury determination only).

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- Concentrated sulfuric acid, AR grade (marked and kept separate for mercury determination only).
- 20% sulfuric acid (v/v): Place 2,400 mL deionized water into a large (4-L) Erlenmeyer flask. Carefully add 600 mL of concentrated sulfuric acid, swirl to mix the solution, and allow it to cool.
- Sulfuric acid:nitric acid mixture (4:1): Mix 4 parts concentrated sulfuric acid with 1 part nitric acid. Carefully swirl to mix the solution.
- 20% hydroxylamine hydrochloride solution: Dissolve 40.0 g in 160 mL deionized water.

Note: Equivalent reagents may be substituted.

PROCEDURE:

1. Weigh a 2- to 4-g sample into an acid-washed, 300-mL round-bottomed flask.

   1.1 Add boiling chips and turn on the cold water for the condenser system.

   1.2 Ensure that the outlet is in the drain and that the pressure is low enough to maintain the system without leakage.

2. Carefully add 25 mL of the sulfuric acid:nitric acid mixture to each of the flasks.

   2.1 Place the flasks in a heating mantle and insert the condensers.

   2.2 Add a stopper to the side arm.

3. Begin heating. If necessary, add very small amounts of nitric acid through the side arm to prevent charring. For example, if the sample begins to darken and there are no brown fumes, add nitric acid. Foaming may occur just before charring. Fats and oils are likely to char.

4. Allow the samples to reflux for 1 hour.

5. Turn off the heat.

6. After cooling the condensers and flasks for about 15 minutes, lightly rinse them with deionized water. Allow them to cool for a few more minutes.

7. Disconnect the condensers and remove the flasks from the heating mantles.

   Note: If desired, start a second batch and monitor it during Steps 8 through 10 of the first batch. Observe Steps 1 through 3 constantly.

8. Add a small amount of saturated potassium permanganate solution to each digested sample and swirl it. Repeat this step until a dark color persists.

9. Quantitatively transfer the digest to an acid-washed, 100-mL volumetric flask. Use deionized water for rinsing.

10. Add 20% hydroxylamine hydrochloride in drops while swirling the solution until the permanganate color disappears. The solution usually becomes colorless, but some other color may remain depending on the nature of the particular sample.

    10.1 Fill the flask to volume with deionized water and mix the solution by inversion.

11. Proceed to the Determination section.

12. After all digestions are complete, turn off the water to the condenser system.

Determination

1. Determine the signal of the standards and samples with an atomic absorption spectrophotometer and a hydride generation unit according to the appropriate IOP.

2. For the instrument variables (wavelength, slit setting, etc.), refer to the "Standard Conditions for Mercury" in the appropriate atomic absorption methods manual.

   2.1 Install the lamp and allow it to warm up. Turn on the recorder and align the cell.

   2.2 Set the operating mode on the spectrophotometer for use with the appropriate (MHS-20) hydride generation unit.

   2.3 Turn on the gas supply (argon).

   2.4 Turn on the power of the MHS-20 controller. Select the $NaBH_4$ mode and adjust the temperature of the cell to 200°C.

   Note: Never use the instrument in the $SnCl_2$ mode when using sodium borohydride.

   2.5 Fill the reservoir with sodium borohydride solution.

3. Purge the system.

   3.1 After the temperature light turns on, set PURGE I to 40 seconds, REACTION to 0 seconds, and PURGE II to 0 seconds.

      3.1.1 Put the empty reaction flask on assembly and push START.

      3.1.2 Wait until the start indicator light turns off, then zero the display.

      3.1.3 Repeat Step 3.1 if necessary.

   3.2 Remove the reaction flask and set PURGE I, REACTION, and PURGE II according to the appropriate hydride generation manual.

4. Read each of the samples and standards using the following techniques.

   4.1 Dispense 10 mL of 20% sulfuric acid solution into the reaction flask and add one drop of the potassium permanganate solution.

      4.1.1 Put the flask on the hydride generation assembly and push START.

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4.1.2 When the start indicator light turns off, remove and empty the reaction flask.

4.1.3 Rinse the flask completely with deionized water.

4.2 Repeat Step 4.1 for all standards and samples. Measure the sample size and dilute the total volume of the reaction to 10.0 mL with 20% sulfuric acid.

4.2.1 Record the aliquot and sample number on the chart.

4.2.2 After analyzing samples with an extremely high mercury content, check for contamination of the system and reaction flask by running a reagent blank.

4.3 Read the standards before and after the group of samples. Use a range of 0.002 to 0.05 µg for each treatment, including 0.002, 0.005, 0.01, 0.02, 0.03, and 0.05 µg.

4.4 Usually read samples using a 10-mL reaction.

4.4.1 If this is too high, take smaller reactions.

4.4.2 If necessary, dilute the sample by diluting an aliquot to volume with 20% sulfuric acid. Then read an appropriate reaction volume from the dilution.

CALCULATIONS:

Perform calculations by preparing a plot of concentration (x-axis) in µg/mL versus signal or absorbance (y-axis). Under the Beer-Lambert Law, the graph is linear (or nearly so), and the concentration of the unknown samples is determined by interpolation.

For computer calculations, enter the raw data (peak heights) and perform calculations by a least squares regression plot of peak height versus concentration.

Formula

$$S = \frac{C \times V \times D}{W \times R}$$

Where:

S = calculated concentration in ppm or µg/L
C = concentration from curve or computer projection in µg
V = volume of sample
W = sample size in g or mL
D = dilution factor
R = reaction volume

Report results using the mnemonic HGE.

APPENDIX D.

Hobe Sound NWR - Mercury/Fish Study, 1991

91-4-4120	Station Location	Species	Total length mm	Total Length inches	Total Weight gm	Total Weight oz	Hg conc. dry wt mg/kg (ppm)	Sample % moisture	Hg conc. wet wt mg/kg (ppm)	Sex
HS-1	Hobe Sound	Gafftopsail Cat	557	21	1555	54	5.498	74.9	1.38	F
HS-2	Hobe Sound	Gafftopsail Cat	542	21	1752	61	4.111	74.7	1.04	F
HS-3	Hobe Sound	Gafftopsail Cat	608	23	2007	70	4.554	77.6	1.02	F
HS-4	Hobe Sound	Gafftopsail Cat	527	20	1425	50	4.714	77.3	1.07	F
HS-5	Hobe Sound	Gafftopsail Cat	652	25	3230	113	6.567	76.7	1.53	F
HS-6	Hobe Sound	Gafftopsail Cat	580	22	2110	74	4.98	75.5	1.22	F
HS-7	Hobe Sound	Gafftopsail Cat	513	20	1460	51	2.651	75.1	0.66	F
HS-8	Hobe Sound	Gafftopsail Cat	603	23	2660	93	5.865	73.4	1.56	F
HS-9	Hobe Sound	Gafftopsail Cat	424	16	678	23	2.08	77.4	0.47	F
HS-10	Hobe Sound	Gafftopsail Cat	547	21	1910	67	2.265	75.5	0.55	M
HS-11	Hobe Sound	Gafftopsail Cat	608	23	2310	81	8.224	74.1	2.13	F
HS-12	Hobe Sound	Gafftopsail Cat	565	22	1825	64	2.789	75.8	0.67	F
HS-13	Hobe Sound	Gafftopsail Cat	594	23	2075	73	5.255	74.5	1.34	F
HS-14	Hobe Sound	Gafftopsail Cat	595	23	2450	86	6.575	74.6	1.67	F
HS-15	Hobe Sound	Gafftopsail Cat	567	22	1856	65	3.318	77.7	0.74	M
HS-16	Hobe Sound	Gafftopsail Cat	580	22	1710	60	6.429	76.2	1.53	F
HS-17	Hobe Sound	Gafftopsail Cat	556	21	1310	46	3.954	76.1	0.95	F
HS-18	Hobe Sound	Gafftopsail Cat	548	21	1962	69	5.84	75	1.46	F
HS-19	Hobe Sound	Gafftopsail Cat	413	16	525	18	3.356	78.4	0.72	F
HS-20	Hobe Sound	Gafftopsail Cat	435	17	710	25	3.253	75.1	0.81	F
HS-21	Hobe Sound	Jack Crevalle	350	13	570	20	2.171	74.2	0.56	
HS-22	Hobe Sound	Jack Crevalle	366	14	630	22	2.465	71.2	0.71	
HS-23	Hobe Sound	Black Drum	377	14	840	29	0.848	77.7	0.19	
HS-24	Hobe Sound	Atlantic Croaker	375	14	375	13	3.873	78.7	0.82	F